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Combined p53 and Bcl2 Immunophenotypes in Prognosis of Vietnamese Invasive Breast Carcinoma: A Single Institutional Retrospective Analysis

Chu Van Nguyen, MD, PhD[1,2], Quang Tien Nguyen, MD, PhD[3], Ha Thi Ngoc Vu, MD[4], Huyen Thi Phung, MD, PhD[5], Khoa Hong Pham, MD, PhD[6], and Roanh Dinh Le, MD, PhD[7]

Abstract

Background: Aberrant of p53 and Bcl2 genes cause changes in the quantity and quality of their proteins and contribute to the pathogenesis of some cancer types including breast cancer. Expression of p53 and Bcl2 were associated to adverse clinical outcomes in breast cancer. **Purpose:** To predict the survival outcomes of invasive breast cancer in Vietnam, using immunohistochemical expression of p53, Bcl2 proteins. **Methods:** The current study was conducted on 526 breast cancer patients who had surgical operations, but had not received neo-adjuvant chemotherapy, from 2011 to 2014. The clinicopathological characteristics were recorded. Immunohistochemical staining was performed on p53, Bcl2 markers. Expression of p53 and Bcl2 were paired into different immunophenotypes for analysis with clinicopathological characteristics and survival. All breast cancer patients' survival were analyzed by using Kaplan-Meier and Log-Rank models. **Results:** The presence of p53 protein was detected in 44.1%. Positive p53, and p53+Bcl2- immunophenotype were significantly associated with poorer prognostic features. In contrast, the positive Bcl2 protein accounted on 57.6%, and combination of p53-Bcl2+ were strong correlated with better clinicopathological parameters. Bcl2 positivity was observed in higher than the negative Bcl2 in the five-year OS (Overall survival) proportion (91.2 vs 79.4%, respectively) ($p < 0.05$). Multivariate analysis revealed that the expression of p53, Bcl2 or combinations of these 2 proteins was no longer remained as an independent prognostic variable. **Conclusion:** The Bcl2 positivity had a distinct OS and DFS (Disease free survival). The expression of p53 and Bcl2 are inversely correlated to clinical outcomes in breast cancer.

Keywords

breast cancer, p53 and Bcl2 immunophenotype, immunohistochemistry, prognosis

Abbreviations

BC, Breast cancer; DFS, Disease free survival; HE, Haematoxylin and eosin staining; IHC, Immunohistochemistry; LUMA, Luminal A; LUMBH, Luminal B HER2(-); LUMBH, Luminal B HER2(+); LVI, Lymph-vascular invasion; NPI, Nottingham Prognostic Index; OS, Overall survival; pCR, pathological Complete Response; TN, Triple negative

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[1] Department of Quan Su Pathology, National Cancer Hospital, Hanoi, Vietnam
[2] Department of Clinical Pathology, Hanoi Medical University, Hanoi, Vietnam
[3] National Cancer Hospital, Hanoi, Vietnam
[4] Vietnam University of Traditional Medicine, Hanoi, Vietnam
[5] Department of Quan Su Internal Medicine, National Cancer Hospital, Hanoi, Vietnam
[6] Department of Quan Su Examination, National Cancer Hospital, Hanoi, Vietnam
[7] Center for Research and Early Detection of Cancer, Hanoi, Vietnam

Corresponding Author:
Chu Van Nguyen, Department of Quan Su Pathology, National Cancer Hospital, 43 Quan Su Street, Hang Bong Commune, Hoan Kiem District, Hanoi, Vietnam.
Email: nguyenvanchu@hmu.edu.vn

Introduction

Breast cancer (BC) is a heterogeneous disease with different tumor subtypes that varies in the prognosis and therapy response. It is clear that the efficacy of systemic adjuvant chemotherapy or endocrine therapy has been demonstrated in BC. Although the improvements of early detection, diagnosis, and treatment, the survival rate is modest, mainly due to the unselective inclusion of patients within a broad group of risk.[1,2] Therefore, evaluating prognostic and predictive biological markers, which are being developed, is still the most important target in BC.[2] Among the prognostic parameters investigated, p53 and Bcl2 genes, and their proteins, received considerable attention as promising prognostic and predictive markers.

At present, many studies demonstrated the role of apoptosis in tumor growth and aggressiveness. The apoptotic process is controlled by numerous genes, including activation of cellular proto-oncogene and lost function of tumor suppressor gene, such as Bcl2 and p53 genes.[3] Aberrant of these genes cause changes in the quantity and quality of their proteins and contribute to the pathogenesis of some cancer types including BC. These genes are involved in growth control and apoptotic pathways, which appear to play a key role in tumor progression in response to anticancer agents, by affecting the magnitude of tumor cell apoptosis or by altering proliferation of tumor cells.[4,5] Bcl2 is a proto-oncogene and encodes the protein Bcl2, which binds to a cell membrane of 26 kDa, inhibits the gene, and regulates cell death according to the program (apoptosis).[5] In BC, Bcl2 is often associated with good clincopathological characteristics such as low mitotic figure, high differentiation, low p53 expression, ER (Estrogen Receptor) positive BC, and good prognostic factors. In many studies, Bcl2 positivity was also associated with a lower risk of recurrence and metastases or better survival rate.[5-14] Meanwhile, p53 abnormalities, a tumor suppressor gene with the molecular weight of 53 kDa, is particularly associated with the pathogenesis of solid tumors such as breast, lung, and colon cancers.[15] Positive p53 often exhibits worse clinical manifestations, such as higher histological grade, negative ER BC, shorter DFS, and OS.[7,9,10,16,17]

For the purpose of improving the survival rate of BC patients, making more suitable decisions on the adjuvant treatment after operating for BC is very important. This requires an essential need for the application of the appropriate treatment protocols, which are used in Vietnam for managing patients with BC.[18] Vietnam is a developing country; hence, the majority of patients with BC could not afford the expensive molecular tests, therefore, selecting the valued and suitable biological markers is crucial, which are appropriate in terms of both expense and value in selecting exactly the adjuvant treatment. Therefore, the current study aimed to predict the survival outcomes of invasive BC in Vietnam, using immunohistochemical expression of p53, Bcl2 proteins.

Material and Methods

Patients and Sample Collection

This is a retrospective study with follow-up of 526 patients with BC aged 14 and 87 years who received an operation, from 2011 to 2014, at the National Cancer Hospital, Vietnam. Next, only treatment—naïve tumors were selected. The patients who presented with second or recurrent malignant tumors were excluded. The clinical information of patients was recorded, such as age, sex, location of tumor, and date at initial diagnosis, which were extracted from medical patient charts and records. Of them, 143 were diagnosed with BC at younger than 45 years old, and the other 69 cases were older than 65. All patients were operated on to remove the tumor by modified radical mastectomy, or conservative surgery, combined with axillary lymph node dissection. Tumors were measured in their maximum diameter. The pTNM staging of BC was staged, basing upon criteria by the American Joint Committee on Cancer (AJCC, seventh edition).[19] Tumor and nodal samples were performed via pathological tests.

After surgery, 477 of the cases were treated by adjuvant chemotherapy. Also, all hormone receptor positive cases were received by endocrine therapy. Among HER2 positive BCs, only 2 patients were able to pay for all expenses for target treatment by trastuzumab. All individual information was deleted or disguised, in order to make sure anonymity was present for the patient.

Histopathology

All specimens were received in the operating room and then transferred to the pathology department. Samples were fixed in 10% neutral formalin for 24 hours. Nodal and tumor samples were obtained by routine pathological techniques, such as haematoxylin and eosin staining. Experienced pathologists evaluated all histopathological features, such as tumor size, histopathological type, grade, nodal status, and peritumoral lymph-vascular invasion (LVI). To confirm lymph-vascular invasion, immunohistochemical staining was used with a D2-40 marker. Histopathological types were classified according to 2012 WHO classifications.[20] Histologic grades were assigned according to Elston and Ellis.[21] The Nottingham Prognostic Index (NPI) was calculated for all breast cancers using the formula: NPI = 0.2 ×tumor size (cm) + lymph node stage (1,2, or 3) + histological grade (1,2, or 3).[22]

Immunohistochemistry and FluoresenceIn Situ Hybridization (FISH)

All immunohistochemistry (IHC) staining was tested for formalin-fixed, paraffin-embedded tissue sections. The IHC method was performed by a Ventana automated machine, using ER, PR, HER2, Ki67, D2-40, p53, and Bcl2 markers. All primary antibodies belonged to Ventana company with a ready to use condition, as the primary monoclonal mouse anti-human estrogen receptor (ER) (Ventana-SP01), anti-progesterone

receptor (PR) (1E2) rabbit monoclonal primary antibody, monoclonal mouse anti-human c-erbB-2 oncoprotein, rabbit monoclonal (Ventana-4B5), confirm anti-Ki67 monoclonal rabbit antibody (Ventana-30-9), podoplanin (D2-40) mouse monoclonal antibody, anti-p53 (DO-7) primary antibody, and anti-Bcl2 (SP66) rabbit monoclonal primary antibody, respectively. The Allred score was used to assess ER and PR status. Breast cancers were scored as ER/PR positive if the total Allred score for ER/PR was >2/8. Now, according to CAP/ASCO guidelines, ER/PR positive was altered to 1%.[23] The UK recommendations were used for assessment of HER2 expression.[22] A HER2 mark of 3 plus was considered HER2 positive, or overexpression. Ninety-six patients (19.2%) who exhibited a IHC HER2 score of 2 plus were tested by FISH to identify amplification of the HER2 gene.[24] 21.9% of HER2 gene amplication occurred by FISH. Numerous different cutpoints for Ki67 were proposed. At the 2013 St Gallen consensus meeting, Ki67 index was divided into 2 levels: low ($\leq$20%) and high (>20%).[25]

All patients were classified into molecular subtypes and risk categories based on age, clinicopathological, and IHC data. Molecular subtypes that follow St Gallen 2013 are Luminal A (LUMA), Luminal B HER2(-) (LUMBH-), Luminal B HER2(+) (LUMBH+), HER2, and Triple-negative (TN).[25] This approach uses IHC criteria for its definition of ER and PR, the detection of HER2 overexpression and/or amplification, and Ki67 index, to identify molecular subtypes. Risk categories were grouped by following St Gallen 2007. Initially, patients were categorized into 3 risk groups: low-risk, intermediate risk, and high-risk, based on the nodal status.[26] After that, more clinicopathological and IHC features were added to this stratification, and the modified versions were published in 2007.[27] Main risk categories of patients with BC were classified as low-risk (LR) (Negative node and all of the features such as pT $\leq$2cm, grade 1, absent LVI, positive ER/or PR, Her2/neu neither IHC overexpression nor amplified, and patients aged $\geq$ 35 years), intermediate risk(IR) (OR Negative node and at least 1 of the features such as pT >2cm, grade 2-3, presence of LVI, ER/or PR negativity, Her2/neu either IHC overexpression or amplified, or patients aged <35 years; EITHER 1-3 positive nodes, and positive ER/or PR, and Her2/neu neither IHC overexpression nor amplified), and high-risk (HR) is defined by OR 1-3 positive nodes and ER/or PR negativity, Her2/neu either IHC overexpression or amplified EITHER 4 or more involved nodes.

P53, Bcl2 IHC Assessment, and Category

All p53 and Bcl2 stained slides were analyzed and scored independently by 2 observers, and discordant were reevaluated to reach consensus. In the present study, among all, 10.1% of cases needed reassessment to resolve the disagreement because various IHC score of the same slide between different pathologists occurred.

The staining locations are consistent with their distribution, such as nuclear envelope, endoplasmic reticulum, and outer mitochondrial membrane for Bcl2 and nuclei for p53. The p53, Bcl2 staining was assessed according to the estimated proportion of nuclear and/or cytoplasmic staining of tumor cells that were positive. Scoring criteria were as follows (in the form of proportion of nuclear and/or cytoplasmic staining = score): absent = 0+, no staining; weakly positive (1+), staining in fewer than 10 percent of the tumor nuclei; moderate (2+), staining in 10% to 75% of tumor nuclei; and strongly positive (3+), staining in more than 75% of tumor nuclei.[28,29] Tumors with a p53 score of 1 or more were considered to be positive for p53 protein accumulation.[29] Meanwhile, Bcl2 scores of 0+ and 1+ were negative, and scores of 2+ and 3+ were positive.[28] The p53 and Bcl2 expression of all patients of the current study were combined into the different prognostic groups, such as p53-Bcl2+ groups (good prognosis), p53+Bcl2+ co-expression (intermediate prognosis), and the negative both as follows: p53-Bcl2- (medium prognosis), and p53+Bcl2- (poor prognosis) (Figure 1).

Follow-up and Outcomes

OS was a period which was defined as the date of initial diagnosis to the day of death, due to BC, or the last available time before losing follow-up.[30] Patients would be censored if they did not die of BC. Death dates were displayed by the death documents, such as certificates, which were issued by the commune government in Vietnam. The recurrence and dates were demonstrated by image analytic and/or morphological data. Patients would be censored until the dead date if they did not present any relapse.[30] DFS was a length of time which was measured as the date of BC surgery until the diagnosis of the recurrent BC or BC specific death, including locoregional and distant relapses.[30] One hundred ninety-six patients with BC were recorded for follow-up information.

Statistical Analysis

To determine the clinicopathological differences between the p53, Bcl2 expression, and categories of both markers, the Pearson chi-square test, Likelihood Ratio, and Fisher's exact tests were performed. The Kaplan-Meier model was used to investigate the five-year OS and DFS, according to the p53, Bcl2 expression, and p53 Bcl2 groups. Survival curves were compared, by performing Log-rank test. A difference will be considered the statistically significance if a p value is less 0.05. In multivariate analysis, Cox proportional hazards regression model were performed to determine hallmarks that were independently associated with OS and DFS. All of the analyses were conducted using the statistical software of SPSS version 19.0.

Results

Correlations of p53, Bcl2 Expression and Clinicopathological Features

The present study was conducted all 526 patients with BC that had undergone operations. Table 1 shows the patients' baseline clinicopathological features and correlations to p53, Bcl2

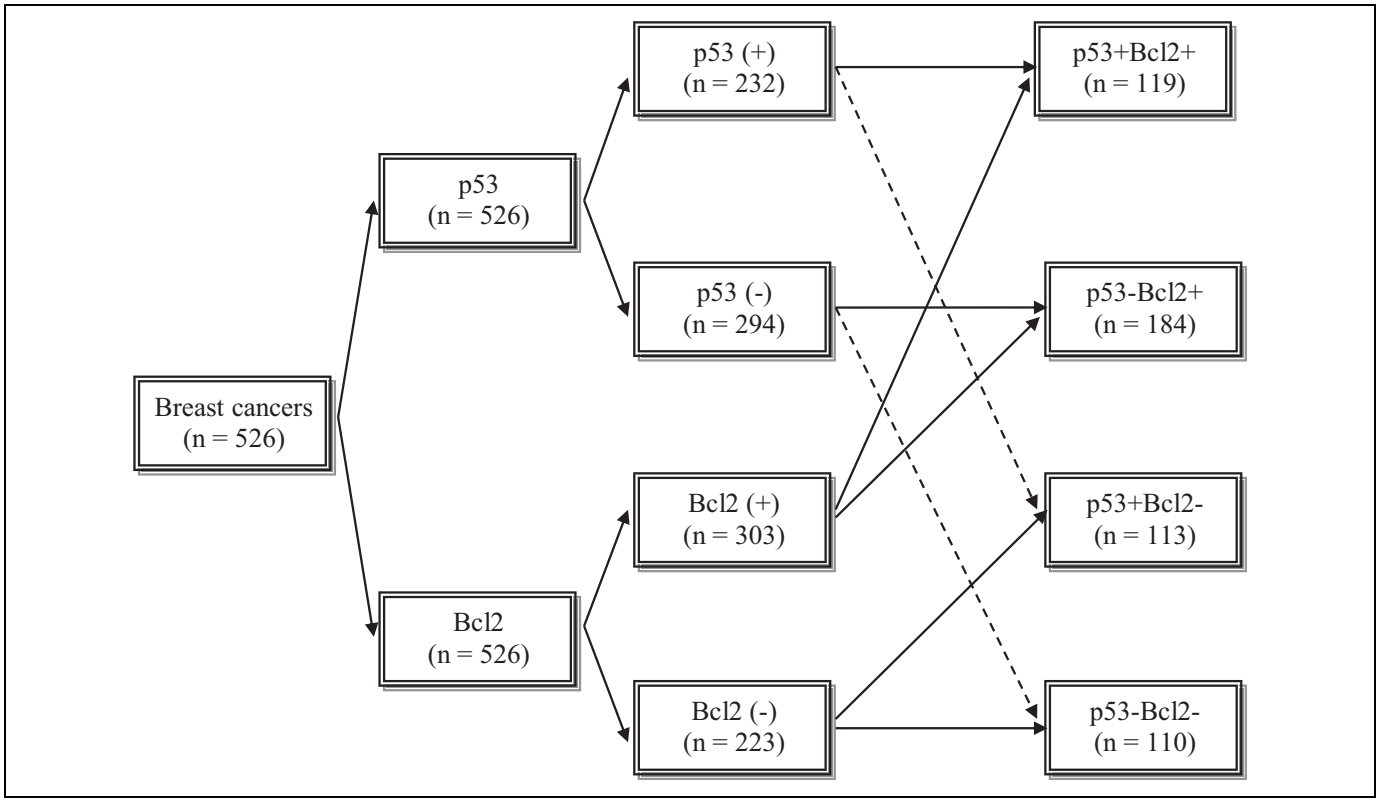

Figure 1. Algorithm of immunophenotypic stratification for p53 and Bcl2 staining.

expression. In 526 patients with BC that were immunohistochemically stained for p53 and Bcl2, the proportion of positive p53 was 44.1% (Figure 2A), and the expression of Bcl2 accounted for 57.6% (Figure 2B). Statistical differences were significantly observed between the positivity of both p53 and Bcl2, with some parameters such as NPI, LVI, hormonal receptor status, HER2, Ki67 index, molecular grouping and risk stratification ($p < 0.05$ or 0.001). In the NPI, p53 expression accounted for a higher proportion in the poor NPI group (27.2%) than the good NPI (14.2%) ($p < 0.05$), while presence of Bcl2 was observed in the good NPI at 24.8%, higher than poor NPI (16.2%) ($p < 0.001$). Positive p53 BC demonstrated a higher LVI rate than those Bcl2 positivity (27.2% vs 19.1%, respectively) ($p < 0.05$).

Regarding hormonal receptor factors (ER, PR), HER2 status, and Ki67 index, more than half of positive Bcl2 BCs were positive, as opposed to present p53 BC ($p < 0.001$). HER2 and a high Ki67 index are 2 prognostic factors for BC that often showed a higher rate in the positive p53 group (34.9 and 63.4%, respectively) than the present Bcl2 breast cancer (16.8 and 42.2%, respectively) ($p < 0.0001$ and 0.05). Additionally, a statistical difference was strong, revealed between risk groups and molecular subtypes in p53 and Bcl2 immunophenotypes ($p < 0.001$). The presence of p53 protein was accounted for a high proportion compared to positive Bcl2 in the poor prognostic molecular subtypes: basal-like type (28.0 vs 15.5%), HER2 type (21.6 vs 5.9%) or HR subgroup (32.8 vs 19.1%), whereas positive Bcl2 breast cancers exhibited a

Table 1. Relationship of p53, Bcl2 Expression and Clinicopathological Features in 526 Operated Breast Cancer Patients.

Characteristics	No. of patients *(%)*	Positive p53 232*(44.1)*	p	Positive Bcl2 303*(57.6)*	p
Age group			0.13		0.032
<40	68*(12.9)*	38*(16.4)*		43*(14.2)*	
40-49	148*(28.1)*	60*(25.9)*		92*(30.4)*	
50-59	190*(36.1)*	88*(37.9)*		92*(30.4)*	
60-69	84*(16.0)*	34*(14.7)*		52*(17.2)*	
≥70	36*(6.8)*	12*(5.2)*		24*(7.9)*	
Young & Older			0.167		0.476
Young (≤45 Y-O)	143*(67.5)*	64*(72.7)*		86*(65,6)*	
Older (≥65 Y-O)	69*(32.5)*	24*(27.3)*		45*(34.4)*	
Lateral			0.171[(a)]		0.541[(a)]
Right	282*(53.6)*	121*(52.2)*		157*(51.8)*	
Left	240*(45.6)*	111*(47.8)*		143*(47.2)*	
Bilateral	4*(0.8)*	0*(0)*		3*(1.0)*	
Tumor size (cm)			0.41		0.018
≤2	224*(42.6)*	92*(39.7)*		144*(47.5)*	
>2-5	283*(53.8)*	130*(56)*		151*(49.8)*	
>5	19*(3.6)*	10*(4.3)*		8*(2.6)*	
Histopathological type			0.356		0.023
NOS	379*(72.1)*	173*(74.6)*		209*(69.0)*	
Lobular	87*(16.5)*	38*(16.4)*		59*(19.5)*	
Mucinous	19*(3.6)*	5*(2.2)*		15*(5.0)*	
Other	41*(7.8)*	16 *(6.9)*		20*(6.6)*	
Histological grade			0.069		0.000
I	55*(10.5)*	17*(7.3)*		40*(13.2)*	
II	189*(35.9)*	81*(34.9)*		125*(41.3)*	
III	282*(53.6)*	134*(57.8)*		138*(45.5)*	

(continued)

Table 1. (continued)

Characteristics	No. of patients (%)	Positive p53 232(44.1)	p	Positive Bcl2 303(57.6)	p
Lymph node status			0.077		0.000
Negative	332(63.1)	134(57.8)		213(70.3)	
1-3 positive node (s)	124(23.6)	63(27.2)		60(19.8)	
>3 positive nodes	70(13.3)	35(25.1)		30(9.9)	
NPI			0.007		0.000
Good	101(19.2)	33(14.2)		75(24.8)	
Moderate	309(58.7)	136(58.6)		178(58.9)	
Poor	116(22.1)	63 (27.2)		49 (16.2)	
LVI			0.044		0.014
Absent	405(77.0)	169(72.8)		245(80.9)	
Present	121(23.0)	63(27.2)		58(19.1)	
ER status			0.000		
Negative	210(39.9)	116(50.0)		67(22.1)	0.000
Positive	316(60.1)	116(50.0)		236(77.9)	
PR status			0.000		0.000
Negative	244(46.4)	133(57.3)		100(33.0)	
Positive	282(53.6)	99(42.7)		203(67.0)	
Her2/neu			0.000		0.000
Negative	393(74.7)	151(65.1)		252(83.2)	
Positive	133(25.3)	81(34.9)		51(16.8)	
Ki67 index			0.000		0.002
Low (≤20%)	274(52.1)	85(36.6)		175(57.8)	
High (>20%)	252(47.9)	147(63.4)		128(42.2)	
Molecular subgroup			0.000		0.000
Luminal A	136(25.9)	29(12.5)		100(33.0)	
Luminal B (HER2-)	137(26.0)	57(24.6)		105(34.7)	
Luminal B (HER2+)	48(9.1)	31(13.4)		33(10.9)	
HER2 positive	85(16.2)	50(21.6)		18(5.9)	
Basal-like	120(22.8)	65(28.0)		47(15.5)	
pTNM stage			0.237		0.000
I	102(19.4)	38(16.5)		77(25.5)	
II	348(66.2)	156(67.5)		193(63.9)	
III	76(14.4)	37(16.0)		32(10.6)	
Risk category			0.001		0.000
Low	19(3.6)	5(2.2)		15(5.0)	
Intermediate	373(70.9)	151(65.1)		230(75.9)	
High	134(25.5)	76(32.8)		58(19.1)	

[a]: Fisher exact test.
[b]: Likelihood Ratio.

higher rate than p53 positivity in luminal A subgroup (33.0 vs 12.5%), luminal B HER2- (34.7 vs 24.6%), or LR category (5.0 vs 2.2%) ($p < 0.05$, and 0.001).

Nevertheless, no statistically significant difference was observed between the expression of both p53 and Bcl2 markers with some parameters of BC in young and old groups, tumor site ($p > 0.05$). For clinicopathological characteristics such as age group, tumor size, histopathological type, histological grade, lymph node status, or pTNM staging, the Bcl2 expression showed a statistically significant difference with these markers ($p < 0.05$ and 0.001); notwithstanding, no difference was noted for the expression of the p53 protein with the above variables ($p > 0.05$).

Clinicopathological Features of Combined p53 and Bcl2 Categories

To evaluate the relationship between clinicopathological features and combined p53 Bcl2 group, p53 and Bcl2 stainings of all patients with BC were paired into 4 groups following expressed status as a combination of p53-Bcl2-, p53+Bcl2-, p53-Bcl2+, and p53+Bcl2+. Table 2 displays the relationship between clinicopathological features and IHC groups of invasive BC. The relationship between the immunophenotypes of combined p53 and Bcl2, with most of the histopathological characteristics, immunophenotypes, molecular subtypes, pTNM stage, and risk classification, revealed a significant difference ($p < 0.05$ and 0.001). Compared to the other phenotypes, absent p53 and Bcl2 positivity (p53-Bcl2+), considering to be a good prognostic group, manifested the highest rate in most prognostic features such as histological grade I (49.1%), negative nodes (40.4%), good NPI (49.5%), absent LVI (3.8%), luminal A (59.6%), stage I (49.0%), LR group (52.6%), and low Ki67 index (45.3%).

On the contrary, the p53+Bcl2- group (considered as the group with poor prognosis) was accounted for a high proportion compared to the remaining groups in some aggressive

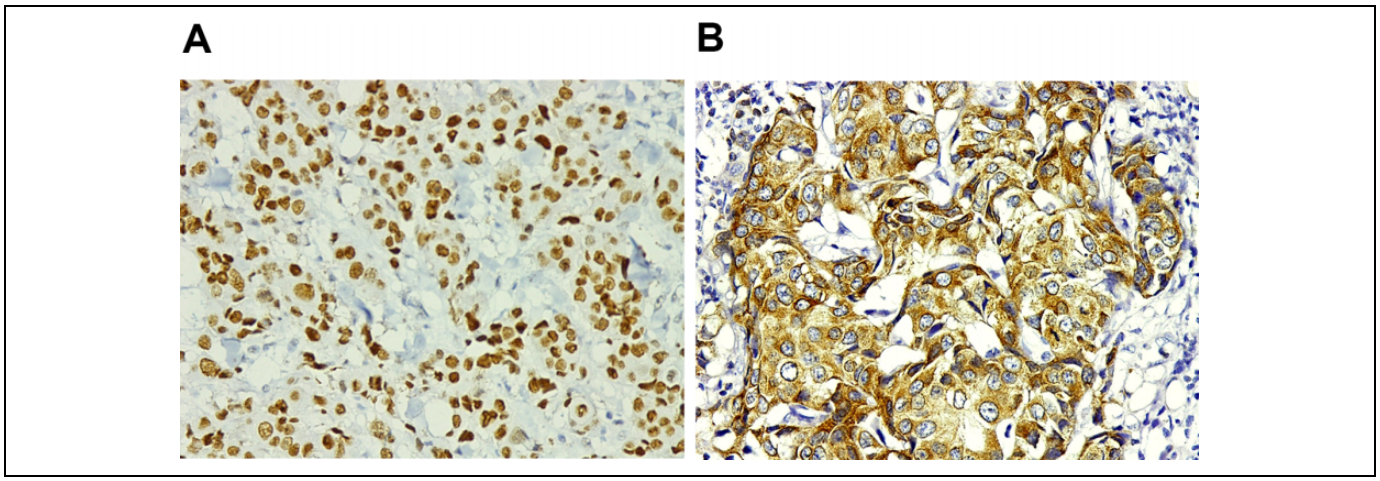

Figure 2. Microscopical pictures illustrated the p53 and Bcl2 expression of invasive BC. (A) Photomicrograph indicated the p53 positive of tumor cell nuclei (IHCx400). (B) Cytoplasm and/or cytoplasm membrane of tumor cells were positive for Bcl2 (IHCx400).

Table 2. Correlation of Combined p53 and Bcl2 to Clinicopathological Characteristics in 526 Operated Breast Cancer Patients.

Characteristics	No. of patients *(%)*	p53 and Bcl2 immunophenotypes				p
		p53-Bcl2- 110 *(20.9)*	p53+Bcl2- 113 *(21.5)*	p53-Bcl2+ 184*(35)*	p53+Bcl2+ 119*(22.6)*	
Age group						0.073
<40	68*(12.9)*	10 *(14.7)*	15 *(21.1)*	20 *(29.4)*	23 *(33.8)*	
40-49	148*(28.1)*	30 *(20.3)*	26 *(17.6)*	58 *(39.2)*	34*(23.0)*	
50-59	190*(36.1)*	44 *(23.2)*	54 *(28.4)*	58 *(30.5)*	34*(17.9)*	
60-69	84*(16.0)*	19 *(22.6)*	13 *(15.5)*	31 *(36.9)*	21*(25.0)*	
≥70	36*(6.8)*	7 *(19.4)*	5 *(13.9)*	17 *(47.2)*	7*(19.4)*	
Young & Older						0.476
Young (≤45 Y-O)	143*(67.5)*	30*(21.0)*	27*(18.9)*	49*(34.3)*	37*(25.9)*	
Older (≥65Y-O)	69*(32.5)*	16*(23.2)*	8*(11.6)*	29*(42.0)*	16*(23.2)*	
Lateral						0.497[(a)]
Right	282*(53.6)*	66*(23.4)*	59 *(20.9)*	95*(33.7)*	62 *(22.0)*	
Left	240*(45.6)*	43*(17.9)*	54 *(22.5)*	86 *(35.8)*	57*(23.8)*	
Bilateral	4*(0.8)*	1*(25.0)*	0*(0)*	3 *(75.0)*	0*(0)*	
Tumor size (cm)						0.110[(a)]
≤2	224*(42.6)*	44*(19.6)*	36*(16.1)*	88*(39.3)*	56 *(25.0)*	
>2-5	283*(53.8)*	61*(21.6)*	71*(25.1)*	92 *(32.5)*	59 *(20.8)*	
>5	19*(3.6)*	5*(26.3)*	6*(31.6)*	4*(21.1)*	4*(21.1)*	
Histopathological type						0.046
NOS	379*(72.1)*	86 *(22.7)*	84 *(22.2)*	120*(31.7)*	89 *(23.5)*	
Lobular	87*(16.5)*	11*(12.6)*	17*(19.5)*	38 *(43.7)*	21*(24.1)*	
Mucinous	19*(3.6)*	4 *(21.1)*	0*(0)*	10*(52.6)*	5 *(26.3)*	
Other	41*(7.8)*	9*(22.0)*	12*(29.3)*	16*(39.0)*	4*(9.8)*	
Histological grade						0.001
I	55*(10.5)*	11*(20.0)*	4*(7.3)*	27*(49.1)*	13 *(23.6)*	
II	189*(35.9)*	32*(16.9)*	32*(16.9)*	76 *(40.2)*	49 *(25.9)*	
III	282*(53.6)*	67*(23.8)*	77 *(27.3)*	81*(28.7)*	57*(20.2)*	
Lymph node status						0.003
Negative	332 *(63.1)*	64*(19.3)*	55 *(16.6)*	134*(40.4)*	79*(23.8)*	
1-3 positive node(s)	124 *(23.6)*	28 *(22.6)*	36*(29.0)*	33*(26.6)*	27 *(21.8)*	
>3 positive nodes	70 *(13.3)*	18 *(25.7)*	22 *(31.4)*	17 *(24.3)*	13*(18.6)*	
NPI						<0.001
Good	101*(19.2)*	18*(17.8)*	7*(7.0)*	50*(49.5)*	26 *(25.7)*	
Moderate	309*(58.7)*	65*(21.0)*	66*(21.4)*	108 *(35.0)*	70 *(22.7)*	
Poor	116*(22.1)*	27*(23.3)*	40*(34.5)*	26 *(22.4)*	23*(19.8)*	
LVI						0.028
Absent	405*(77.0)*	83*(20.5)*	77*(19.0)*	153*(37.8)*	92 *(22.7)*	
Present	121*(23.0)*	27*(22.3)*	36*(29.8)*	31*(25.6)*	27*(22.3)*	
ER status						
Negative	210*(39.9)*	64*(30.5)*	79 *(37.6)*	30*(14.3)*	37 *(17.6)*	<0.001
Positive	316*(60.1)*	46*(14.6)*	34 *(10.8)*	154*(48.7)*	82*(25.9)*	
PR status						<0.001
Negative	244*(46.4)*	64*(26.2)*	80 *(32.8)*	47*(19.3)*	53*(21.7)*	
Positive	282*(53.6)*	46*(16.3)*	33*(11.7)*	137*(48.6)*	66*(23.4)*	
Her2/neu						<0.001
Negative	393*(74.7)*	77*(19.6)*	64 *(16.3)*	165*(42.0)*	87*(22.1)*	
Positive	133*(25.3)*	33*(24.8)*	49*(36.8)*	19*(14.3)*	32*(24.1)*	
Ki67 index						<0.001
Low (≤20%)	274*(52.1)*	65*(23.7)*	34*(12.4)*	124*(45.3)*	51*(18.6)*	
High (>20%)	252*(47.9)*	45 *(17.9)*	79 *(31.3)*	60*(23.8)*	68 *(27.0)*	
Molecular subgroup						<0.001
Luminal A	136*(25.9)*	26 *(19.1)*	10 *(7.4)*	81 *(59.6)*	19 *(14.0)*	
Luminal B (HER2-)	137*(26.0)*	16*(11.7)*	16 *(11.7)*	64*(46.7)*	41 *(29.9)*	
Luminal B (HER2+)	48*(9.1)*	6*(12.5)*	9 *(18.8)*	11*(22.9)*	22 *(45.8)*	
HER2 positive	85*(16.2)*	27*(31.8)*	40*(47.1)*	8*(9.4)*	10*(11.8)*	
Basal-like	120*(22.8)*	35*(29.2)*	38 *(31.7)*	20*(16.7)*	27*(22.5)*	
pTNM stage						0.001
I	102*(19.4)*	14 *(13.7)*	11*(10.8)*	50 *(49.0)*	27*(26.5)*	
II	348*(66.2)*	77*(22.1)*	77 *(22.1)*	114 *(32.8)*	80*(23.0)*	
III	76*(14.4)*	19*(25.3)*	24*(32.0)*	19*(25.3)*	13*(17.3)*	
Risk						<0.001[(a)]
Low	19 *(3.6)*	4*(21.1)*	0*(0)*	10*(52.6)*	5*(26.3)*	
Inter	373*(70.9)*	76 *(20.4)*	67 *(18.0)*	146 *(39.1)*	84 *(22.5)*	
High	134*(25.5)*	*30 (22.4)*	46*(34.3)*	28 *(20.9)*	30 *(22.4)*	

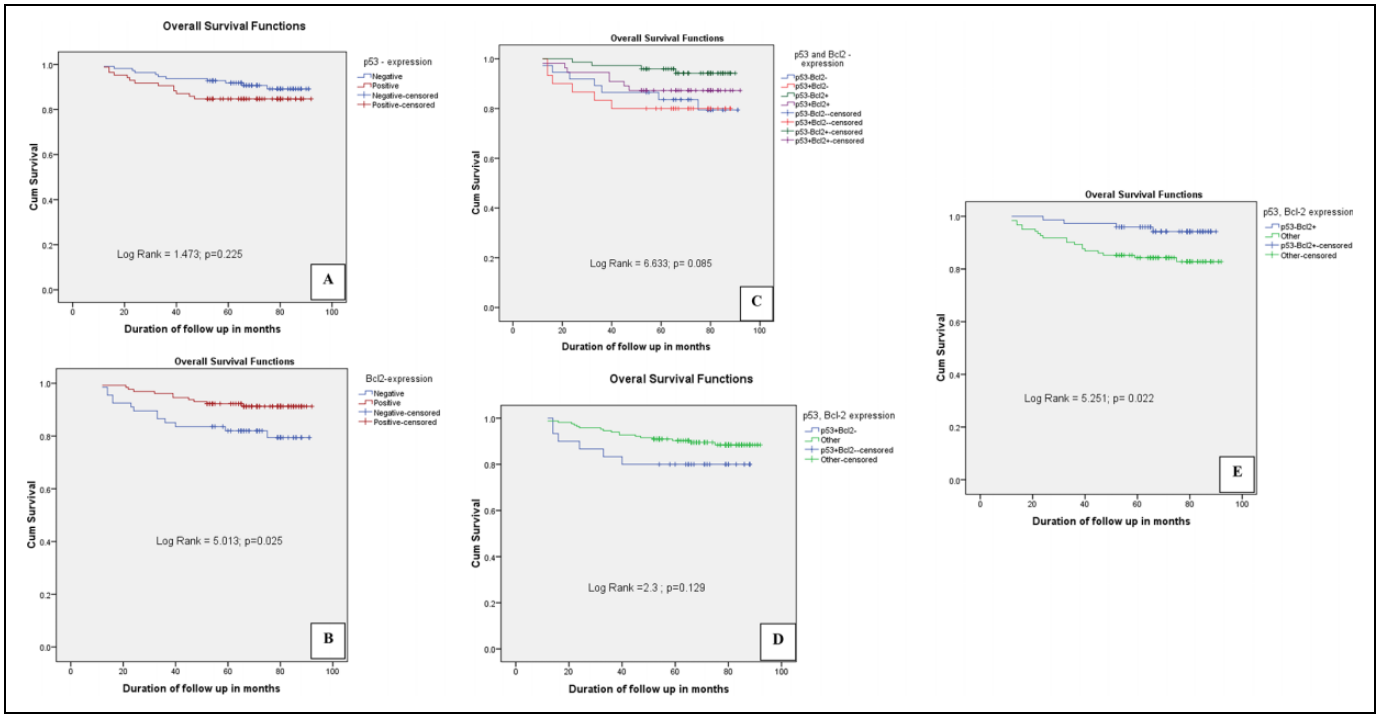

Figure 3. (A) Five-year relative overall survival of expression of p53 protein for invasive breast cancers. The Log-rank test exhibits that there is not a significant difference between these 2 survival curves. (a) Photomicrograph indicated the p53 positive of tumor cell nuclei (x400). Five-year relative overall survival of expressed Bcl2 for infiltrating breast cancers. The Log-rank test indicates that there is a significant difference between the 2 survival curves. (C) Five-year relative overall survival of combination of expressed p53 and Bcl2 in invasive breast cancers. The Log-rank test shows that there was not a significant difference between these 4 survival curves. (D) OS of the p53+Bcl2- immunophenotype compared to the remaining groups, the Logrank test shows that there was not a significant difference between these 2 survival curves. (E) A significant difference between the 2 survival curves of the p53-Bcl2+ and the other immunophenotypes was observed.

characteristics, such as more 3 metastatic lymph nodes (31.4%), poor NPI (34.5%), LVI (29.8%), basal-like type (31.7%), stage III (32.0%), HR category (34.3%), HER2+ (36.8%), and high nucleic proliferation (31.3%).

Regarding features of age group, young and older BC, neoplastic site, tumor size, a significant difference was not demonstrated in p53 and Bcl2 IHC groups ($p > 0.05$). Both negative p53 and Bcl2, or co-expressed p53 and Bcl2 immunophenotypes tended to get higher proportion than p53+Bcl2-, but they were lower than p53-Bcl2+ group in the good prognostic clinicopathological features such as grade I, negative node, good NPI, absent LVI, negative HER2, low Ki67, stage I, or low risk category.

Survival

The medium five-year OS of the operated on patients with BC was 84.65 $\pm$ 1.45 months, in which the negative and positive p53 were 85.65 $\pm$ 1.62 and 82.24 $\pm$ 2.55 months, respectively; by contrast, the lack, and presence of Bc2 was 79.03 $\pm$ 3.10 and 87.19 $\pm$ 1.43 months, in turn. For p53 and Bcl2 categories, the mean five-year OS of p53-Bcl2+ group displayed the highest (87.39 $\pm$ 1.33 months); on the contrary, p53+Bcl2- category demonstrated the lowest (75.10 $\pm$ 4.78 months), meanwhile, p53+Bcl2+ and p53-Bcl2- were counted in the medium range (84.65 $\pm$ 1.45 and 80.34 $\pm$ 3.87 months, respectively). OS curves according to p53 expression are summarized in Figure 3A. Patients who were negative for p53 protein demonstrated the better prognosis, with an OS rate in the five-year follow-up of 89.1%. Patients with p53 positivity got the lower five-year OS rate (84.7%). However, a statistically significant difference was not found ($p > 0.05$). Figures 3B demonstrates that difference was statistically significant between OS curves, according to Bcl2 protein ($p < 0.05$). Presence of Bcl2 was observed in higher than the negative Bcl2 in the five-year OS proportion (91.2 vs 79.4%, respectively). The OS curves, according to p53 Bcl2 categories, are illustrated in Figure 3C. Patients who were in the p53-Bcl2+ category revealed the best prognosis, with an OS rate in the five-year follow-up of 94.2%; by the contrast, p53-Bcl2- and p53+Bcl2- groups exhibited in the lowest OS rate (79.4 and 80.0%, respectively), and co-expression group (p53+Bcl2+) accounted in the intermediate OS proportion (87.3%). Nevertheless, a statistical significance was not observed in these differences ($p > 0.05$). Regarding the p53+Bcl2- phenotype compared to the remaining phenotypes, Figure 3D showed that the 5-year survival of patients with p53+Bcl2- was lower than other phenotypic breast cancers, 80.0 vs 88.4%, respectively. However, this adverse trend was not observed with statistical significance ($p > 0.05$). In contrast,

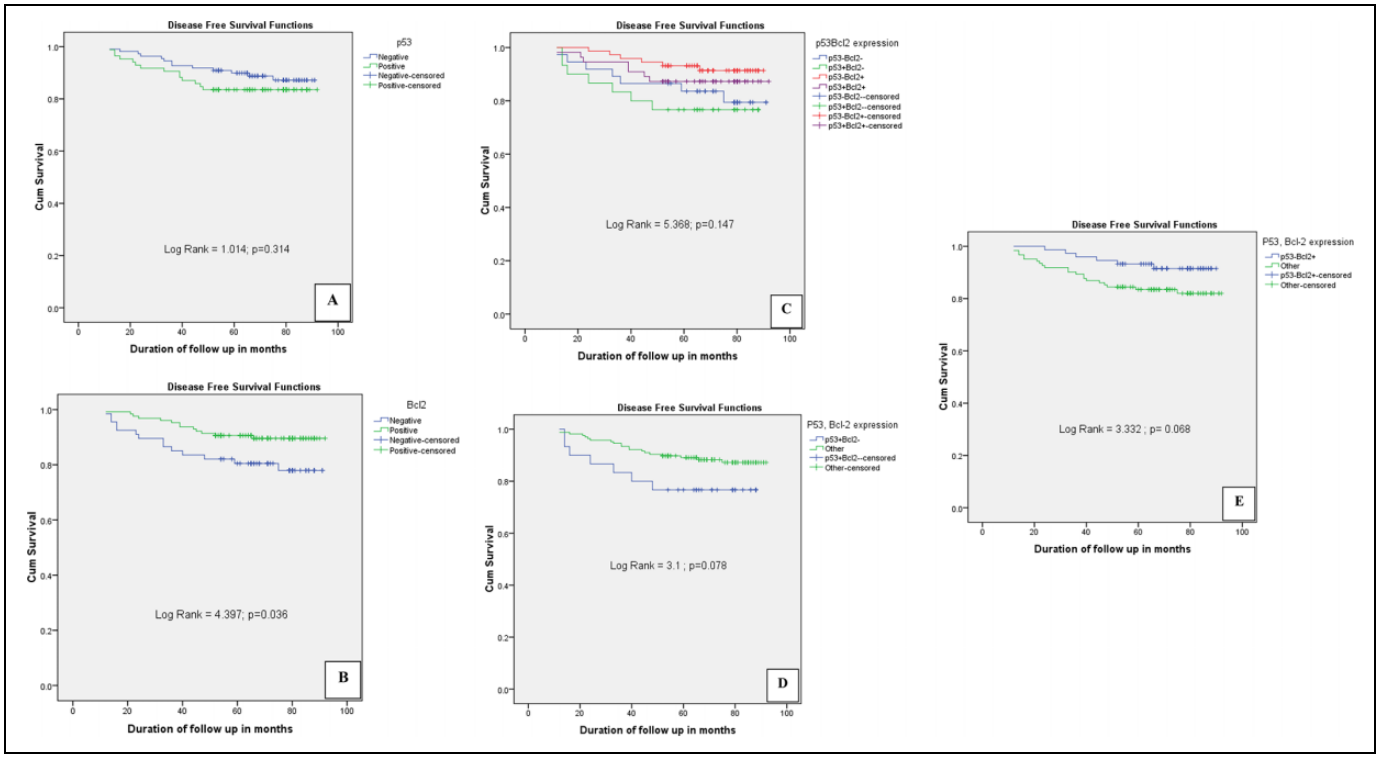

Figure 4. Five-year relative disease free survival of p53 positivity and negativity in invasive breast cancers. The Log-rank test demonstrates that there was not a significant difference between these 2 survival curves (A). Microscopical picture was stained by IHC stain, tumor cells were positive for Bcl2 (b) (x400). Chart B revealed a significant difference between 2 survival curves of five-year relative disease free survival of Bcl2 expression for invasive breast cancers. The Log-rank test displayed that there was not a significant difference between 4 and five-year relative disease free survival curves of combination of p53 and Bcl2 expression for infiltrating breast cancers (C). Significant differences were also not demonstrated between the DFS curves of the p53+Bcl2-, and the p53-Bcl2+ and other immunophenotypes (D and E, respectively).

observing the p53-Bcl2+phenotype with the remaining phenotypes, the demonstrated difference was statistically significant ($p < 0.05$), and the 5-year survival rate of patients with p53-Bcl2+ was clearly higher than the breast cancers with other phenotypes, it was 94.2 and 82.8%, respectively (Figure 3E).

Mean DFS was 83.85 $\pm$ 1.51 months. Medium DFS for positive p53 was lower than Bcl2 positivity, with 81.72 $\pm$ 2.58 and 86.85 $\pm$ 1.51 months, respectively. For combined p53 and Bcl2 immunophenotypes, the moderate five-year DFS of p53-Bcl2+ group showed the highest (85.98 $\pm$ 1.62 months), and by contrast, the p53+Bcl2- category displayed the lowest (73.77 $\pm$ 4.84 months), meanwhile, p53+Bcl2+ and p53-Bcl2- were counted on the medium level, similarly to whose OS. DFS curves according to different markers are shown in Figure 4A, B, C, D and E. A significant difference was observed in the 5-year DFS rate, according to DFS and Bcl2 ($p = 0.036 < 0.05$) (Figure 4A). The DFS rates in the five-year follow-up of the patients with the Bcl2 positivity were higher than lack of Bcl2 (89.6% vs 78.0%). On the contrary, for p53 protein, a difference was not statistically significant in the five-year DFS ($p = 0.31 > 0.05$) (Figure 4B). Positive p53 was counted on 83.5%, lower than p53 negativity (87.2%) in the five-year DFS proportion. The DFS curves according to the combined p53 and Bcl2 categories were illustrated in Figure 4C. Patients who were in the p53-Bcl2+ category demonstrated the best prognosis, with a DFS rate in the five-year follow-up of 91.4%; by the contrast, p53+Bcl2- group exhibited in the lowest DFS proportion (76.7%, respectively). The co-expressed p53 and Bcl2 group (p53+Bcl2+) and the lack of both p53 and Bcl2 group (p53-Bcl2-) were ranged in the intermediate DFS proportion between p53-Bcl2+and p53+Bcl2- immunophenotypes (87.3 and 79.4%, respectively). Nevertheless, these differences were not observed with a statistical significance ($p > 0.05$). Regarding the p53+Bcl2- BCs with other immunophenotypes, Figure 4D displayed that the DFS prevalence of the p53+Bcl2- group was lower than the other groups (76.7 and 87.2%, respectively). Conversely, BCs with the p53-Bcl2+ phenotype were shown to exhibit higher DFS than the other immunophenotypes (91.5 and 82.0%, respectively) (Figure 4E). Although, the difference was not observed the statistically significances ($p > 0.05$).

Identifying the independent prognostic significance of clinicopathological variables and expression of IHC markers, multivariate analysis was performed, and showed in Table 3. Only parameters with a significant result ($p < 0.05$) in univariate impact were applied in the multivariate analysis, such as hormonal receptors, Her2/neu status, mitotic proliferation, NPI, differentiated grade, LVI, lymph node condition,

Table 3. Estimated Hazard Ratios (HRs) for OS and DFS—Multivariate Analysis.

	Overall survival		Disease free survival	
	HR	p-value	HR	p-value
p53 status Positive vs negative	0.691	0.412	0.748	0.488
Bcl2 status Positive vs negative	0.486	0.165	0.232	0.562
Immunophenotype p53+Bcl2- vs other	0.495	0.459	0.870	0.873
ER status Positive vs negative	1.171	0.84	1.252	0.761
PR status Positive vs negative	1.695	0.5	1.433	0.62
Her2/neu status Positive vs negative	0.816	0.677	0.755	0.536
Ki67 index High vs low	1.865	0.183	1.923	0.133
NPI Poor vs other	0.802	0.726	0.984	0.979
Histological grade Grade III vs other	0.576	0.314	0.504	0.172
LVI Present vs absent	0.352	0.025	0.427	0.05
Lympho node status >3 vs 0-3 node (s)	0.469	0.225	0.465	0.209
Risk group High vs other	2.857	0.142	2.377	0.199
Molecular subgroup Luminal A vs other	0.000	0.942	0.000	0.931
pTNM Stage III vs other	1.468	0.549	1.592	0.461

molecular subtype, risk category, and pTNM. Nevertheless, p53, Bcl2 markers, and their combination were not demonstrated as an independent prognostic indicator. LVI was the unique factors considered to be a parameter of the independent prognosis ($p < 0.05$).

Discussion

The tumor suppressor p53 gene encodes for a 53kDa nuclear phosphoprotein located in nuclei.[15] Whereas, the proto-oncogene Bcl2 encodes a mitochondrial membrane protein placing in envelope of nuclei, endoplasmic reticulum, and outer mitochondrial membrane.[5] Two genes are involved in multifunction of cell cycle proliferative control and programmed cell death. Both their proteins are related to pathways of the programmed cell death and provide prognostic information on breast cancer.[5,15,31] The presence of mutant p53 may be linked to loss of Bcl2 and a subsequent synergistic increase in cellular proliferation.[10] The goal of most cancer treatments is to reduce cellular proliferation or increase programmed cell death.[9] The expression of p53 and Bcl2 genes as a positive and negative regulator of apoptosis can often be functionally altered in cancer cells, and their proteins are the factors of the significant prognosis in BC. In most of the previous investigations, the presence of p53 protein has been shown to exhibit a worse outcome. It is associated with high histological grade, negative ER, a poor response to chemotherapy, and shorter disease free survival (DFS) and overall survival (OS).[7,9,10,16,17] Otherwise, Bcl2 positivity has been correlated with favorable prognostic features in BC, such as positive ER, low proliferative index, differentiated tumor grade, lower recurrent risk and distant metastases, or better OS.[5-14]

Until now, the expression of the p53 and Bcl2 markers has mainly been investigated in breast cancer, separately. In Vietnam, this is the first time that we have evaluated the combination of these 2 markers to form 4 different immunophenotypes, in order to provide more useful information for the treatment and prognosis of breast cancer. The results of the present study demonstrated a significant contrast between the combined p53 and Bcl2 immunophenotypes with some clinicopathological characteristics. Positive p53 or p53 positivity and lack of Bcl2 was significantly associated with poorer prognostic features, such as high NPI, LVI, negative hormonal receptor BC, positive Her2/neu, high Ki67, basal-like subtype, or HR category. In contrast, the presence of Bcl2 protein or absence of p53 and Bcl2 positivity was strong correlated with better clinicopathological parameters, such as small tumor size, low histological grade, less lymph node metastasis and lymphatic invasion, low NPI, breast cancers with hormonal receptorpositive, negative Her2/neu, low proliferative activity, common in luminal A subgroup, LR level, and early-staged BC. The present study also found that an expression of p53, or the combined p53+Bcl-2, tends to make the OS and DFS shorter. Bcl2 expression, or negative p53 and positive Bcl2, increased OS and DFS significantly. Likewise, Dawson's study revealed that Bcl2 positivity continued to be associated with a favorable prognostic effect with long-term follow-up.[11] According to the research of Malamou-Mitsi et al., their results showed that positive p53 is a negative prognostic factor of OS and DFS. Bcl2 exhibits no effect on the OS or DFS.[7] The current findings illustrated a significant adverse association between the expression of p53 and Bcl2, and their combined immunophenotypes in BC, although in multivariate analysis, have not been shown to be independent prognostic factors. The results are consistent with previous studies.[6,7,10,31,32]

Bcl2 has been widely demonstrated in preventing the apoptosis induced by drugs of chemotherapy in cancer cell lines.[33] Positivity of p53 was strong correlated to pCR in patients with TNBC after neoadjuvant chemotherapy.[34] Combination of these 2 markers was studied by several investigators. Studies revealed a correlation between the p53+Bcl2- phenotype and higher grade and the p53-Bcl2+ phenotype and lower grade invasive ductal cancers.[35,36] Regarding the evaluation of the effect on the OS and DFS by a combination of 2 proteins, the current findings displayed that lack of p53 expression and Bcl2 positivity (p53-Bcl2+) tended to increase OS and DFS, especially the significantly increased OS. By contrast, positive p53 and lack of Bcl2 expression exhibited a trend to reduce survival, both of OS and DFS. It is similar to the findings of Rolland.[10] Mdzinet al.also demonstrated an inverse correlation

of p53 and Bcl2 protein expressions, and the combined Bcl2 overexpression and loss of p53 are useful markers in predicting good prognostic outcome in patients with BC.[6] P53-Bcl2+ increased OS, but they were not the independent prognostic markers in multivariate analysis;it is similar to other factors. Whereas, a combination of p53 and lack of Bcl2 expression (p53+Bcl2-) are associated with reduced 5 year survival, and in multivariate analysis, it was still an independent and poor prognostic factor to other parameters.[10] The current study is consistent with results of previous studies. Considering the ratio of immunophenotypes of p53 and Bcl2 to OS and DFS, we found that these rates decreased gradually in the following order: p53-Bcl2+ > p53+Bcl2+ > p53-Bcl2-/p53+Bcl2-. These findings were suitable with our initial hypothesis. This confirms the prognostic role of the pair of p53 and Bcl2 markers in breast carcinoma, although Bcl2 or p53 was not an independent prognostic factor.

In the performed multivariate analysis, additional prognostic factors revealed that the effect of Bcl2 on survival is no longer statistically significant, although the positivity of Bcl2 significantly increased both OS and DFS in univariate analysis. In several studies, Bcl2 was not demonstrated to be an independent prognostic power for DFS and OS.[6,37] However, it was only a factor of an independent prognosis in a subgroup of patients with positive nodes in multivariate analysis.[37] Especially in some previous studies, results showed that the elevated expression of Bcl2 was an independent prognostic indicator for poorer OS, as such a significant marker for tumor aggressiveness, and a predictive role of resistance to chemotherapy in TNBC.[17,31,34] In contrast, the findings of study's Dawson displayed that Bcl2 was an independent parameter.[11] Similarly, Honma et al. illustrated that Bcl2 positivity is an independent poor prognostic power in patients with negative hormonal receptor or TNBCs, especially in the absence of adjuvant therapy.[14] In the present study, LVI was the unique factors considered to be a parameter of the independent prognosis, in the multivariate analysis. Separating the real LVI from stromal artifact is very important. To accurately determine the LVI, all BC samples of this study were stained by D2-40 IHC maker. Also, this factor provides valuable information for therapy decision-making and prognosis.

In breast cancer, the precious mechanism of differential Bcl2 protein expression is complex. Bcl2 is expressed in normal epithelium of breast, and Bcl2 exhibits a positive correlation with ER alpha (Erα) status. This raised the possibility that Bcl2 is an ER-regulated gene, an ability as a direct result of transcriptional induction.[38-40] Patients with BC with ER+/Bcl2- were found to exhibit a worse prognostic than those with ER-/Bcl2+ BC. The interaction between treatment and the prognostic role of Bcl2 was also addressed, showing that the prognostic impact of Bcl2 is the independence of adjuvant therapy received.[11] The favorable prognosis previously observed in positive Bcl2 cancer seems to reflect the indirect effect of frequently co-expressed hormone receptors and adjuvant endocrine therapy.[14]

Limitations of the Study

At present, some limitations still remained in the present study. Not all patients were followed up. Some reasons to explain this include the fact that the patient database was not being systematically managed on the computer system, and, moreover, patients exhibit a tendency to change their phone numbers in Vietnam. Therefore, keeping in contact with them when they completed their treatment was challenging. Continued follow-up and analysis of all patients are planned to confirm the prognostic value of combination of p53 and Bcl2 in BC. Only 2 HER2 positive patients received anti-HER2 therapy. This is due to the majority of Vietnamese patients being poor, and insurance companies do not cover all expenses of this therapy. Therefore, their families cannot pay for all regimen of the trastuzumab treatment. If all these patients received the target treatment, their survival rate would have been improved better.

Conclusions

The p53, Bcl2 expression and their combined immunophenotypes in BC were adverse correlated to clinical characteristics, and particularly, Bcl2 expression and p53-Bcl2+ exhibited a distinct OS and DFS. The current findings suggest that the p53, Bcl2 expression, and especially their combination, could be used to provide the valuable information for treatment and prognosis of Vietnamese patients with BC.

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Author Contributions

C.V.N, Q.T.N, H.T.P, and K.H.P equally major contributed to this work.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Considerations

All objects of the protocol's this study was approved by the Ethical Committee of National Cancer Hospital, Vietnam as number: 2027/BVK-HDDD. Written informed consent was applied to all patients before enrolling them to the study. Patients could withdraw from the study at any time without any threats or disadvantages and for no stated reasons.

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ORCID iD

Chu Van Nguyen https://orcid.org/0000-0001-8928-5089

References

1. Aapro SM. Adjuvant therapy of primary breast cancer: a review of key findings from the 7th International Conference St Galen February 2001. *The Oncologist*. 2001;6(4):376-385.
2. Marić P, Ozretić P, Levanat S, Oresković S, Antunac K, Beketić-Oresković L. Tumor markers in breast cancer: evaluation of their clinical usefulness. *Coll Antropol*. 2011;35(1):241-247.
3. Greenblab MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res*. 1994;54(18):4855-4878.
4. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science*. 1991;253(5015):49-53.
5. Lu QL, Abel P, Foster CS, Lalani EN. bcl-2: role in epithelial differentiation and oncogenesis. *Hum Pathol*. 1996;27(2): 102-110.
6. Mdzin R, Lau TY, Rohaizak M, Sharifah NA. Inverse correlation between P53 and Bcl-2 expression in breast carcinoma of Malaysian patients. *Med & Health*. 2012;7(1):32-40.
7. Malamou-Mitsi V, Gogas H, Dafni U, et al. Evaluation of the prognostic and predictive value of p53 and Bcl-2 in breast cancer patients participating in a randomized study with dose-dense sequential adjuvant chemotherapy. *Ann Oncol*. 2006;17(10): 1504-1511.
8. Jager JJ, Jansen RLH, Arends JW. Clinical relevance of apoptotic markers in breast cancer not yet clear. *Apoptosis*. 2002;7(4): 361-365.
9. Kharrat AF-, Bouraoui S, Rahal K, Gamoudi A, May MVE. *al e.* P53 and Bcl 2 expression in breast cancer. Prospective study in Tunisia. *Autral-Asian J Cancer*. 2003;2(2):79-82.
10. Rolland P, Spendlove I, Madjd Z, et al. The p53 positive Bcl-2 negative phenotype is an independent marker of prognosis in breast cancer. *Int J Cancer*. 2007;120(6):1311-1317.
11. Dawson SJ, Makretsov N, Blows FM, et al. BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. *Br J Cancer*. 2010;103(5):668-675.
12. Laura M, Vargas-Roiga F, Cuello-Carrióna DO, et al. Prognostic value of Bcl-2 in breast cancer patients treated with neoadjuvant anthracycline based chemotherapy. *Mol Oncol*. 2008;2(1): 102-111.
13. Silvestrini R, Veneroni S, Daidone MG, et al. The Bcl-2 protein: a prognostic indicator strongly related to p53 protein in lymph node-negative breast cancer patients. *J Natl Cancer Inst*. 1994; 86(7):499-504.
14. Honma N, Horii R, Ito Y, et al. Differences in clinical importance of Bcl-2 in breast cancer according to hormone receptors status or adjuvant endocrine therapy. *BMC Cancer*. 2015;15: 698.
15. Steele RJ, Thompson AM, Hall PA, Lane DP. The p53 tumour suppressor gene. *Br J Surg*. 1998;85(11):1460-1467.
16. Beenken SW, Grizzle WE, Crowe DR, et al. Molecular biomarkers for breast cancer prognosis: coexpression of c-erbB-2 and p53. *Ann Surgery*. 2001;233(5):630-638.
17. Ozretic P, Alvir I, Sarcevic B, et al. Apoptosis regulator Bcl-2 is an independent prognostic marker for worse overall survival in triple-negative breast cancer patients. *Int J Biol Markers*. 2018; 33(1):109-115.
18. Chu NV, Quang NT, Ha VTN, et al. Application of St Gallen categories in predicting survival for patients with breast cancer in Vietnam. *Cancer Control*. 2019;26(1):1-10.
19. Fleming ID, Greene FL, Page DL, et al. *AJCC Cancer Staging Manual, 7th edition*. Springer-Verlag; 2010.
20. Lakhani SR, Elis IO, Schnitt SJ, et al. *WHO Classification of Tumors of the Breast*. IARC; 2012.
21. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*. 1991;19(5):403-410.
22. NHS. *Cancer Screening Programmes Jointly With The Royal College of Pathologists. Pathology Reporting of Breast Disease: Joint Document Incorporating the Third Edition of the NHS Breast Screening Programme's Guidelines for Pathology Reporting in Breast Cancer Screeningand the Second Edition of The Royal College of Pathologists' Minimum Dataset for Breast Cancer Histopathology*. NHSBSP Publication; 2005.
23. Hammond MEH, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists Guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch Pathol Lab Med*. 2010;134(6):907-922.
24. Wolff DJ, Bagg A, Cooley LD, et al. Guidance for fluorescence in situ hybridization testing in hematologic disorders. *J Mol Diagn*. 2007;9(2):134-143.
25. Untch M, Gerber B, Harbeck N, et al. 13th St. Gallen International Breast Cancer Conference 2013: primary therapy of early breast cancer evidence, controversies, consensus—opinion of a German team of experts (Zurich 2013). *Breast Care*. 2013;8(3):221-229.
26. Goldhirsh A, Glick JH, Gelber RD, Senn HJ. Meeting highlights: international consensus panel on the treatment of primary breast cancer. *J Natl Cancer Inst*. 1998;90(21):1601-1609.
27. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thürlimann B, Senn HJ. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer. *Ann Oncol*. 2007;18:1133-1144. doi:10.1093/annonc/mdm271
28. Bertolo CP, Guerrero D, Vicente F, et al. Differences and molecular immunohistochemical parameters in the subtypes of infiltrating ductal breast cancer. *Am J Clin Pathol*. 2008;130(3): 414-424.
29. Yamashita H, Nishio M, Toyama T, et al. Coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer. *Breast Cancer Res*. 2004;6(1):R24-R30.
30. Choudhury KR, Yagle KJ, Swanson PE, Krohn KA, Rajendran JG. A robust automated measure of average antibody staining in immunohistochemistry images. *J Histochem Cytochem*. 2010; 58(2):95-107.
31. Bonetti A, Zaninelli M, Leone R, et al. Bcl-2 but not p53 expression is associated with resistance to chemotherapy in advanced breast cancer. *Clin Cancer Res*. 1998;4(10):2331-2336.

32. Tsutsui S, Yasuda K, Suzuki K, et al. Bcl-2 protein expression is associated with p27 and p53 protein expressions and MIB-1 counts in breast cancer. *BMC Cancer*. 2006;13(6):187.
33. Ohmori T, Podack ER, Nishio K. Apoptosis of lung cancer cells caused by some anti-cancer agents (MMC, CPT-11, ADM) is inhibited by bcl-2. *Biochem Biophys Res Commun*. 1993; 192(1):30-36.
34. Kim T, Han W, Kim MK, et al. Predictive significance of p53, Ki-67, and Bcl-2 expression for pathologic complete response after neoadjuvant chemotherapy for triple-negative breast cancer. *J Breast Cancer*. 2015;18(1):16-21.
35. Lee WY, Jin YT, Tzeng CC. Reciprocal expression of Bcl-2 and p53 in breast ductal carcinoma. *Anticancer Res*. 1996;16(5A): 3007-3012.
36. Fabi A, Mottolese M, Benedetto AD, et al. p53 and BLC2 immunohistochemical expression across molecular subtypes in 1099 early breast cancer patients with long-term follow-up: an observational study. *Clin Breast Cancer*. 2020;20(6):e761-e770.
37. Hellemans P, Dam PAV, Weyler J, Oosterom ATV, Buytaert P, Marck EV. Prognostic value of bcl-2 expression in invasive breast cancer. *Br J Cancer*. 1995;72(2):354-360.
38. Wang TT, Phang JM. Effects of estrogen on apoptotic pathways in human breast cancer cell line MCF-7. *Cancer Res*. 1995; 55(12):2487-2489.
39. Leung LK, Wang TT. Paradoxical regulation of Bcl-2 family proteins by 17 beta-oestradiol in human breast cancer cells MCF-7. *Br J Cancer*. 1999;81(3):387-392.
40. Doglioni C, Tos APD, Laurino L, Chiarelli C, Barbareschi M, Viale G. The prevalence of Bcl-2 immunoreactivity in breast carcinomas and its clinicopathological correlates, with particular reference to estrogen receptor status. *Virchows Arch*. 1994; 424(1):47-51.